Sustained Delivery of Stabilized Proteins from Electrospun Tissue Scaffolds

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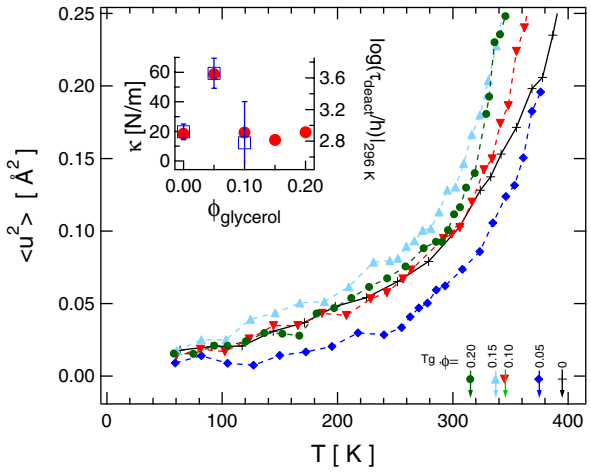
It is generally accepted that integral gene or drug delivery systems will feature in successful tissue scaffolds for regenerative medicine. These may serve to foster proliferation and direct differentiation and expression of extracellular matrix.

Proteins or genetic material are often mixed directly with the biodegradable polymers that make up a scaffold. While this approach has thus far led to successful efforts in timed release of proteins, it places some restrictions on the formulation parameters that can be accessed. For example, the protein is often mixed with scaffold polymer in the presence of organic solvents, and this can sometimes harm (deactivate) a more labile protein. Furthermore, a dry environment of protein or polymer is not necessarily conducive to extended stability of proteins at physiological temperatures.

Glasses composed partly of sugars are utilized in nature to preserve labile biological structures in low-moisture situations. Such sugar glasses have been used widely for stabilization of pharmaceutical proteins. These glasses have the advantage that they can be formulated from water, and thus are gentler on the biologically active molecules to be delivered.

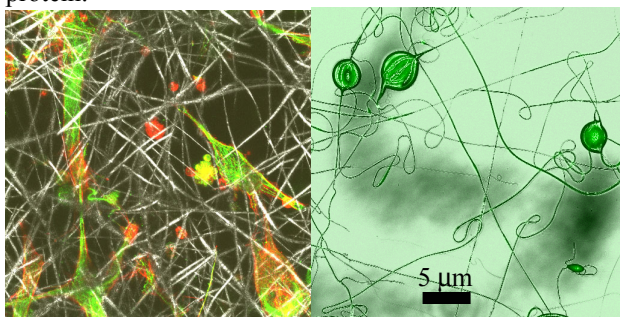
We have established methods to aid in *a priori* screening of materials for stabilization of proteins, for nano-encapsulation of proteins in those selected materials, and for incorporation of the stabilized proteins into tissue scaffolds for programmed release.

Some glasses are significantly better for preserving proteins than others, whether they are sugar glasses or biodegradable polymers. We have shown that suppressed fast, local dynamics is an important characteristic for effective preservant glasses. The figure below shows Debye-Waller factors ($\langle u^2 \rangle$), obtained as a function of temperature by inelastic neutron scattering, for a series of trehalose glasses. We produced the series by diluting the trehalose glasses with glycerol in varying mass fractions (ϕ).



The symbols (+, ♦, ▼, ▲, ●) indicate glycerol mass fractions of 0, 0.05, 0.1, 0.15, and 0.2 respectively. The insert shows a clear correlation between stability (deactivation time, or τ_{deact}) of horseradish peroxidase (HRP) in these glasses and the temperature dependence of $\langle u^2 \rangle$, labelled "κ". Symbols in the inset (● and □) represent measurements of κ and HRP stability, respectively. Error bars represent standard uncertainties of ± 1 standard deviation. These measurements are general, and can also be used for proteins in biodegradable scaffolds.

Pharmaceutical glasses can be used to beneficial effect as an aid to incorporating proteins in tissue scaffolds. Stabilizing the protein relaxes constraints on solvents that can be used. We show that glasses made of good biopreservation sugars and / or water-soluble polymers can also protect proteins from harsh organic solvents that may otherwise denature them. The micrograph below on the left shows a polycaprolactone electrospun tissue scaffold hosting chondrocytes; on the right are similar electrospun fibres that have small spheres of a protein-bearing glass incorporated in them. The fibres were spun out of acetone / isooctane with no adverse effect to the protein.



Smaller particles of protein-bearing glass than shown above are desirable. Such particles can be produced by freeze-drying inverse micelles of dioctyl sulfosuccinate, with the aqueous phase composed of the protein and a glass former. We have produced freeze-dried particles of 70 nm average diameter, containing on average about 10^3 protein molecules each. The particles appear to be clusters of inverse micelles each bearing 0.75 HRP molecules on average. Proteins encapsulated this way appear to be more stable even than those in bulk pharmaceutical glass.

We show that sustained delivery of *active* protein can be achieved for a model protein, HRP, from an electrospun tissue scaffold under physiological conditions for a period of at least thirty days.